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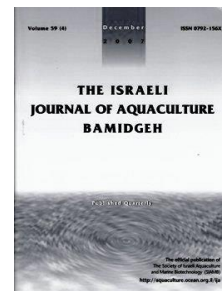
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Effects of Soya Saponins on Feed Intake, Growth Performance, and Cholesterol Metabolism in Juvenile Turbot (*Scophthalmus maximus* L)

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Abstract

An 8-week feeding trial was conducted to investigate the effects of soya saponins on feed intake, growth performance, and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L).

A control diet and two experimental diets were prepared with the supplementation of soya saponins, concentrations being as follows: 0.0% (Diet 1, Control), 0.25% (Diet 2) and 0.5% (Diet 3), respectively. The results showed that 0.25% of dietary soya saponins produced comparable growth performance with the control diet. However, the growth performance of fish fed the diet with 0.5% soya saponins was significantly lower. The selected parameters measured in plasma, liver, and feces of each group were not significantly different. These results suggested that 0.25% dietary soya saponins did not produce negative effects, but 0.5% of dietary soya saponins significantly reduced fish growth. Dietary soya saponin supplementation to FM-based diet did not significantly affect cholesterol metabolism.

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Introduction

Soybean meal (SBM) has been widely used as the most cost-effective alternative protein source for fish meal (FM) in fish feeds (Refstie et al., 2000; Chou et al., 2004; Wang et al., 2006). Growth performance was not affected in Black Sea Turbot (*Psetta maeotica*) when fishmeal was partially replaced with defatted soybean (Ergun et al., 2008) but was lowered in many carnivorous fish species fed SBM-based diets, e.g. rainbow trout (*Oncorhynchus mykiss*) (Refstie et al., 2000), cobia (*Rachycentron canadum*) (Chou et al., 2004), cuneate drum (*Nibea miichthioides*) (Wang et al., 2006) and sharpnose seabream (*Diplodus puntazzo*) (Hernández et al., 2007). The detrimental effect may be due to antinutritional factors (ANFs) in SBM which contains trypsin inhibitors, lectins, antivitamin, soy antigens, phytic acid, and saponins (Francis et al., 2001a; Gatlin III et al., 2007). The trypsin inhibitors, lectins, and antivitamin are heat sensitive and may be destroyed by thermal treatment; antigenic compounds and phytate levels in fish diets are usually unlikely to affect fish growth performance (Francis et al., 2001a).

Soya saponins are triterpenoid glycosides and can be divided into two major groups: A and B (Knudsen et al., 2008). They derive from a number of plant species in leaves, stem, seeds, bark, blossoms, fruit, and roots (Francis et al., 2001c). SBM usually contains approximately 0.43–0.67% soya saponins (Ireland et al., 1986; Bureau et al., 1998). The effects of dietary soya saponins on growth response of fish are inconsistent. A significant reduction in growth and intestinal damage of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout was observed when fish were fed a diet containing 0.3% soya saponins (Bureau et al., 1998). However, there was no significant difference in feed intake and growth of channel catfish (*Ictalurus punctatus*) when fed the diet with 0.26% purified soya saponins (97% of soya saponin B) (Twibell and Wilson, 2004). Dietary *Quillaja* saponins at a rate of 0.015% showed potential for growth promotion in common carp (*Cyprinus carpio* L.) (Francis et al., 2001b) and tilapia (*Oreochromis niloticus*) (Francis et al., 2001c).

The hypocholesterolemic effect was observed in plasma of many fish species fed plant-based diets compared with fish fed FM-based diet. The species studied include turbot (*Psetta maxima*) (Regost et al., 1999), gilthead sea bream (*Sparus aurata*) (Gomez-Requeni et al., 2004; Venou et al., 2006), Atlantic cod (*Gadus morhua* L.) (Hansen et al., 2007), rainbow trout (Kaushik et al., 1995; Yamamoto et al., 2007), and parrot (*Oplegnathus fasciatus*) (Lim and Lee, 2009). The hypocholesterolemic effect has also been observed in plasma of turbot (*Scophthalmus maximus* L.) fed plant-based diets compared with fish fed FM-based diet (Yun et al., 2011).

Turbot is a carnivorous species widely cultured in Europe and East Asia because of its high quality flesh and rapid growth. Dietary effects of soya saponins have not been reported on FI and growth performance of juvenile turbot. It is also unknown if and how dietary soya saponins affect cholesterol metabolism in fish fed FM-based diet. Therefore, the aim of this study was to investigate the effects of soya saponins on feed intake, growth performance, and cholesterol metabolism in juvenile turbot fed FM-based diet.

Materials and Methods

Feed Ingredients and Diet Formulation. Soya saponins (84.96% purity) were obtained from North China Pharmaceutical (Shijiazhuang, China) Co., Ltd. The remaining soya saponins were mainly soybean protein (8.26%) and soybean oligosaccharide (4.77%) according to the supplier. Using FM as the primary protein source, fish oil and soybean oil as the lipid sources, and wheat flour as the carbohydrate source, a control diet (Diet 1) with 61% FM was formulated. The other two isonitrogenous and isolipidic experimental diets were prepared with supplementation of 0.3% and 0.6% soya saponins (84.96% purity), respectively. Composition of the diets is given in Table 1. The planned soya saponin concentrations in the diets were 0.0%, 0.25% and 0.5% respectively. L-threonine, L-arginine, L-valine, L-isoleucine, L-leucine and L-phenylalanine (Crystalline amino acids, CAAs) were supplemented to meet the essential amino acids (EAA) requirements based on whole body amino acid profile of juvenile turbot (Kaushik, 1998).

Table 1. Formulation (% diet), proximate composition (% diet) and energy content (MJ /kg) of the experimental diets

Ingredients	Diet 1	Diet 2	Diet 3
Fish meal ^a	61	61	61
Wheat flour	28.95	28.65	28.35
Fish oil	2	2	2
Soybean oil	4	4	4
Soybean lecithin	1	1	1
Vitamin premix ^b	0.8	0.85	0.85
Mineral premix ^b	0.5	0.5	0.5
Amino acid premix ^c	1.65	1.65	1.65
Ethoxyquin	0.0	0.05	0.05
Soya saponins product ^d	0.0	0.3	0.6
<i>Analyzed nutrients compositions (on dry matter basis %)</i>			
Soya saponins ^e	0.0	0.25	0.5
Dry matter	90.47	91.29	93.70
Crude protein	52.52	52.31	52.32
Crude lipid	12.51	11.94	12.40
Gross energy	20.9	20.99	21.00
Ash	9.19	9.57	9.52

^a Fish meal: steam-dried fishmeal (COPENCA Group. Lima, Peru)

^b Yun et al. (2011)

^c Amino acid premix (g/kg diet): L-arginine 5.0, L-valine 3.0, L-threonine 1.0, L-isoleucine, 2.0, L-leucine, 3.0, L-phenylalanine, 2.5.

^d Soya saponins product (84.96% pure) was obtained from North China Pharmaceutical (Shijiazhuang, China) Co., Ltd. The remaining of soya saponins product was mainly soybean protein (8.26%) and soybean oligosaccharide (4.77 %) according to the supplier.

^e The value was calculated according to soya saponins product (84.96% purity).

Ingredients were ground into fine powder and sieved through a 246- μ m mesh. The soya saponins were first dissolved in deionized water, mixed with the ingredients thoroughly, and then mixed with fish oil, and then water to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China), and dried for about 12 h in a ventilated oven at 45°C, and then stored at -20°C until use.

Fish, Experimental Conditions and Samples Collection. Juvenile turbot were obtained from Yellow sea fisheries Co., Ltd (Haiyang, Shandong, China). Fish were acclimated to the system and fed the control diet for 2 weeks before the trials. Juvenile turbot (initial body weight: 5.91 \pm 0.02 g) were randomly distributed into 9 flat-bottomed, constantly aerated, tanks filled with 300 l sea water which was continuously pumped from the coast adjacent to the experiment station, passed through sand filters into each tank at approximately 1.5 l/min. Three replicate tanks were assigned to each diet group and 60 fish of similar size were bulk weighed and stocked in each tank. During the 8-week feeding period, fish were fed the experimental diets to apparent satiation twice daily at 07:00 and 18:00. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70°C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying and reweighing. At the end of the experiment, 5 fish from each tank were sampled for morphometric parameters. Individual body weight, body length, liver weight and visceral weight were recorded to calculate condition factor, hepatosomatic index, and viscerosomatic index. All experimental fish were anesthetized with Eugenol (1:10000) (Shanghai Reagent Corporation, Shanghai, China) before sampling. Blood samples were taken from the caudal vein using heparinized syringes. Plasma samples were prepared after centrifugation (4000 g for 10 min) at 4°C and immediately stored at -80°C until analysis. Liver samples were frozen in liquid nitrogen and stored at -80°C for subsequent determination of lipid content. The feces collection method was the same as described in Deng et al. (2010).

During the 8-week feeding period, water temperature was controlled at 19-22°C, pH 7.5-8.0, salinity 30-33‰, ammonia nitrogen was lower than 0.1 mg/l, nitrite was lower than 0.1 mg/l, and dissolved oxygen was higher than 6.0 mg/l.

Chemical analysis Dry matter, crude protein, crude lipid, ash, and gross energy were analyzed for content and influence of experimental diets (AOAC, 1995). Dry matter was analyzed by drying the samples to constant weight at 105°C. Crude protein was determined through digestion using the Kjeldahl method and estimated by multiplying nitrogen by 6.25 (N \times 6.25). Crude lipid was measured by ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550°C for 16 h. Gross

energy was determined by Parr 1281 Automatic Bomb Calorimeter (Parr, Moline, IL, USA). Duplicate analyses were conducted for each sample.

The concentration of total cholesterol (TC), free cholesterol (FC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in plasma were determined by colorimetric enzymatic methods using commercial kits (FC kit supplied by Shanghai Mingdian Bioengineering Co., Shanghai, China; other kits supplied by Changchun Huili bio-Tech Co., Changchun, China) (Yun et al., 2011). After extraction of the lipids from 500 mg liver, and feces with chloroform: methanol (2:1, v/v) (Folch et al., 1957), the TC content in liver and feces, and FC content in liver were determined using the same kits as for the plasma. The volume of lipid solution was made to 10 ml with chloroform: methanol (2:1, v/v). One milliliter of this extract was sampled, dried under a pure nitrogen stream, and the obtained residue was mixed with 1 ml isopropyl alcohol containing 100 g Triton X-100/I (Reagent Grade) (Yun et al., 2011). The amount of cholesterol esters in plasma and liver was calculated by subtracting the FC value from the TC value. Duplicate analyses were conducted for each sample.

Calculations and statistical methods. Growth parameters were calculated as follows: Weight gain rate, WGR (%) = $100 \times [(\text{final body weight} - \text{initial body weight}) / \text{initial body weight}]$.

Feed intake, FI (%/day) = $100 \times \text{total amount of the feed consumed (g)} / [(\text{initial body weight} + \text{final body weight}) / 2] / \text{days}$.

Feed efficiency ratio, FER = $\text{weight gained (g)} / \text{total amount of the feed consumed (g)}$.

Survival rate, SR (%) = $100 \times (\text{final fish number} / \text{initial fish number})$.

Condition factor, CF = $100 \times \text{fish weight} / (\text{body length})^3$.

Hepatosomatic index, HSI (%) = $100 \times (\text{liver weight} / \text{body weight})$.

Viserosomatic index, VSI (%) = $100 \times (\text{visceral weight} / \text{body weight})$.

Software SPSS, 11.5 was used for all statistical evaluations. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when $P < 0.05$. All data are expressed as means \pm standard error (S.E).

Results

All experimental fish showed high survival rate (>99%) (Table 2), and no significant difference was observed among dietary treatments ($P > 0.05$). Growth performance in fish fed FM-based diet with 0.25% soya saponins (Diet 2) was not inhibited, and FI and FER were not significantly affected compared with fish fed Diet 1 (Control). However, fish fed the diet with 0.5% soya saponins (Diet 3) showed significantly lower final body weight (FBW), WGR and FER than fish fed the control diet ($P < 0.05$, Table 2), but FI was not significantly affected.

Table 2. Growth performance and survival rate of turbot fed the experimental diets (Means \pm SE, n = 3)*

	Diet 1	Diet 2	Diet 3
IBW ¹	5.89 \pm 0.0	5.92 \pm 0.01	5.92 \pm 0.01
FBW ²	32.7 \pm 0.2 ^b	32.3 \pm 0.6 ^b	30.4 \pm 1.3 ^a
WGR ³	455.0 \pm 3.8 ^b	445.3 \pm 9.5 ^b	412.9 \pm 21.0 ^a
FER ⁴	1.30 \pm 0.0	1.32 \pm 0.03	1.24 \pm 0.03 ^a
FI ⁵	1.88 \pm 0.0	1.91 \pm 0.05	1.88 \pm 0.01
SR ⁶	100 \pm 0.0	99.4 \pm 0.6	100 \pm 0.0

¹ IBW: initial body weight.

² FBW: final body weight.

³ Weight gain rate (WGR) (%) = $100 \times [(\text{FBW} - \text{IBW}) / \text{IBW}]$.

⁴ Feed efficiency ratio (FER) = $(\text{FBW} - \text{IBW}) / \text{total amount of the feed consumed}$.

⁵ Feed intake (FI) (%/day) = $100 \times \text{total amount of the feed consumed} / [(\text{IBW} + \text{FBW}) / 2] / \text{days}$.

⁶ Survival rate (SR) (%) = $100 \times (\text{final fish number} / \text{initial fish number})$.

*Values in the same row with different superscripts are significantly different ($P < 0.05$).

Table 3. Condition factor, hepatosomatic index and viserosomatic index of turbot fed the experimental diets (Means \pm SE, n = 3)*

	Diet 1	Diet 2	Diet 3
CF ¹	3.30 \pm 0.03	3.28 \pm 0.12	3.37 \pm 0.08
HSI ²	1.41 \pm 0.06	1.39 \pm 0.13	1.32 \pm 0.08
VSI ³	5.40 \pm 0.15	5.20 \pm 0.05	5.15 \pm 0.06

¹ Condition factor, CF = $100 \times \text{fish weight} / (\text{body length})^3$.

² Hepatosomatic index, HSI (%) = $100 \times (\text{liver weight} / \text{body weight})$.

³ Viserosomatic index, VSI (%) = $100 \times (\text{visceral weight} / \text{body weight})$.

*Values in the same row with different superscripts are significantly different ($P < 0.05$).

CF, HSI and VSI of turbot at the end of growth trial are presented in Table 3. HSI (from 1.32 to 1.41) and VSI (from 5.15 to 5.40) of fish given increasingly greater dietary soya saponins, showed a decreasing trend but no statistical differences were observed among dietary treatments with respect to CF, HSI and VSI ($P>0.05$).

Plasma TC (4.13–4.66 mmol/L), FC (2.27–2.62 mmol/L), cholesterol esters (1.51–2.11 mmol/L), HDL-C (2.99–3.13 mmol/L), LDL-C (1.87–1.93 mmol/L) and HDL-C/LDL-C (1.52–1.65) after 24 h starvation in each group were not significantly different ($P>0.05$, Table 4). No significant differences were detected among dietary treatments with respect to TC (3.30–3.92 g /kg wet liver), FC (2.47–3.24 g/kg wet liver) and cholesterol esters (0.68–0.86 g/kg wet liver) in liver ($P>0.05$, Table 5). No statistical difference was observed between the dietary treatments ($P>0.05$) in TC (5.83–6.37 g/kg dry matter) in feces, (Table 5).

Table 4. Cholesterol concentrates in plasma of turbot fed the experimental diet (mmol/L, Means \pm SE, n = 3)*

	Diet 1	Diet 2	Diet 3	
Total cholesterol	4.66 \pm 0.13	4.13 \pm 0.79	4.36 \pm 0.50	¹ HDL-C: high-density lipoprotein cholesterol;
Free cholesterol	2.55 \pm 0.36	2.62 \pm 0.31	2.27 \pm 0.12	² LDL-C: low-density lipoprotein cholesterol.
Cholesterol esters	2.11 \pm 0.28	1.51 \pm 0.71	2.09 \pm 0.52	*Values in the same row with different
HDL-C ¹	3.13 \pm 0.35	2.99 \pm 1.18	3.13 \pm 0.97	superscripts are significantly different
LDL-C ²	1.92 \pm 0.20	1.93 \pm 0.54	1.87 \pm 0.39	($P<0.05$).
HDL-C/LDL-C	1.64 \pm 0.15	1.52 \pm 0.09	1.65 \pm 0.09	

Table 5. Lipid profiles in liver (g/kg wet liver) and feces (g/kg dry matter) of turbot fed the experimental diet (Means \pm SE, n = 3)*

	Diet 1	Diet 2	Diet 3
Liver total cholesterol	3.30 \pm 0.90	3.92 \pm 0.61	3.70 \pm 0.51
Liver free cholesterol	2.47 \pm 0.66	3.24 \pm 0.70	2.84 \pm 0.62
Liver cholesterol esters	0.83 \pm 0.27	0.68 \pm 0.54	0.86 \pm 0.17
Feces total cholesterol	6.11 \pm 0.41	5.83 \pm 1.04	6.37 \pm 0.13

*Values in the same row with different superscripts are significantly different ($P<0.05$).

Discussion

Usually, SBM contains approximately 0.43–0.67% soya saponins (Ireland et al., 1986; Bureau et al., 1998). This data was used as a reference for experimental diets designed for many fish species (Bureau et al., 1998; Twibell and Wilson, 2004; Knudsen et al., 2008; Chen et al., 2011). However, the reported effects of supplemental dietary soya saponins on growth responses of fish differed. In the present study, growth performance in turbot fed FM-based diet with 0.25% soya saponins (Diet 2) was not depressed compared with fish fed Diet 1 (Control). This result was similar with that of Chen et al. (2011) where growth of Japanese flounder (*Paralichthys olivaceus*) fed 0.32% soya saponins added to their diet containing 68% FM was not depressed. Similar results have also been obtained in channel catfish fed casein-based diets (Twibell and Wilson, 2004). However, Bureau et al. (1998) concluded that 0.3% *Quillaja* saponins added to salmonid feed containing 32% of SPC caused significant intestinal damage, and depressed growth of chinook salmon and rainbow trout. These differences could be due to different contents of soya saponins, and different protein sources used in diets. It has been reported that 0.16% soya saponins added to feed containing 62% of FM neither depress growth nor induce intestinal damage of Atlantic salmon, but fish fed the diet with 0.17% soya saponins in combination with lupin kernel meal displayed significant enteritis (Knudsen et al. 2008).

A high level of soya saponins (0.64%) greatly depressed the growth performance of Japanese flounder fed FM-based diet (Chen et al., 2011). Similarly, in this study, turbot fed the diet with 0.5% soya saponins (Diet 3) significantly depressed growth compared with the control diet. This suggests that saponin levels (0.5%) have a deleterious effect as they exceed the endurance limitation of turbot even if fish are fed a FM-based diet. Saponins have been implicated in reduced feed intake and resultant reduced growth by imparting an undesirable taste to the feed (Dersjant-Li, 2002). In the present study, FI of fish fed diet with 0.5% soya saponins (Diet 3) was not affected. This result suggests

that the deleterious effects of soya saponin supplementation in diet, on fish may not be attributed to FI. In this study, fish fed the diet with 0.5% soya saponins (Diet 3) showed significantly lower FER than that of fish fed the control diet ($P < 0.05$). Moreover, the mechanism of these deleterious effects of soya saponins in diet on fish is possibly related to impairment of distal intestinal histology, apparent digestibility coefficients of nutrients, and subsequently reduction of feed efficiency ratio (Chen et al., 2011).

In the present study, hypocholesterolemic effect was not observed in the plasma of fish fed diets with 0.25% or 0.5% soya saponins as compared with that of fish fed the control diet. Furthermore, no significant differences were detected among dietary treatments with respect to FC and cholesterol esters in plasma.

Cholesterol metabolism is related to uptake, biosynthesis, transport, and export (Maita et al., 2006; Yun et al., 2011). The main cholesterol synthesis (endogenous cholesterol) was conducted in the liver of fish (Maita et al., 2006). In this study, no significant differences were detected between dietary treatments with respect to TC, FC and cholesterol esters in liver. It is well known that LDL carries cholesterol from the liver to peripheral tissues, while HDL carries cholesterol from peripheral tissues to the liver (Chen et al., 2003; Deng et al., 2010). Therefore, HDL-C/LDL-C ratio could be used as a marker of cholesterol transport. In the present study, there were no statistical differences in plasma HDL-C, LDL-C and HDL-C/LDL-C levels in turbot fed diets with 0.25% or 0.5% soya saponins compared with fish fed the diet without soya saponin supplementation (control diet), which indicated that cholesterol transport was not significantly affected by the supplemented dietary soya saponins. Feces cholesterol (0.583–0.637% dry matter) was not significantly affected by dietary soya saponins. The present study suggests that dietary soya saponin supplementation did not show any effect on cholesterol metabolism of juvenile turbot fed a FM-based diet. This result is the first reported in fish.

In conclusion, results of the present study showed that a diet with 0.25% soya saponins did not significantly affect fish growth performance. However, fish fed the diet with 0.5% soya saponins suffered significantly depressed growth and feed utilization, but had no significant effect on FI. Moreover, dietary soya saponin supplementation to FM-based diet had no significant effect on cholesterol metabolism in fish.

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